# Seed Production of White Clover Cultivars and Naturalized Populations when Grown in a Pasture

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## ABSTRACT

**Small-type white clover, *Trifolium repens* L., plants predominate in most closely grazed pastures in the southeastern USA. The role of relative seed production in stand persistence of white clover types in pastures has not been quantified. This study compared the relative seed production of seven small-type naturalized populations with that of seven large-type white clover cultivars and germplasms in a pasture. All entries were space-planted into plots in a common bermudagrass [*Cynodon dactylon* (L.) Pers.] pasture at Mississippi State, MS, on a Savannah fine sandy loam (fine-loamy, siliceous, semiactive, thermic Typic Fragiudult) in fall 1995 and 1996, and grazed with cattle prior to flowering and seed production. All naturalized populations averaged about three times as many flowers and seed-bearing flowers as all cultivars other than 'Louisiana S-1' each year. Flower production differences were consistent throughout the study, though maximum flower production for naturalized populations was earlier in the season than cultivars. Seed production differences were similar to flower production, as cultivars averaged only 27 to 43% as much seed as naturalized populations. The low growth stature and excessive seed production of naturalized populations enable them to tolerate close continuous grazing and have a greater opportunity to reseed in pastures than common large-type cultivars. This reseeding potential probably contributes to the domination of small-type white clover in closely grazed pastures of the southeastern USA.**


Most U.S. white clover breeding efforts during the last 50 yr have resulted in the release of large-type or ladino white clovers (Pederson, 1995). In the southeastern USA, these releases have included cultivars such as 'Regal', 'Osceola', 'Tillman', and 'Will' and germplasms SRVR and Brown Loam Syn. 2 (Caradus and Woodfield, 1997). Advantages of large-type over intermediate or small-type white clover include greater plant size, greater initial productivity, and improved summer persistence due to larger, thicker stolons. Since large-type cultivars have been the predominant type planted in southeastern USA grazing systems, it might be expected that most volunteer plants found in pastures would be large-type white clover. However, this is not the case. In most closely grazed pastures, small-type white clover predominates. This could be due to a number of factors, including grazing avoidance or tolerance, superior stolon branching and rooting, flower production, pest or disease resistance, or reseeding ability (Harris, 1987; Brink et al., 1999). Widdup et al. (1996) found that only 10 of 98 ecotype collections from throughout the eastern USA were ladino-type plants. Bouton et al. (1998) reported that ecotype collections in Georgia were all intermediate types, even in pastures previously planted with large-type cultivars. All white clover plants collected from 27 closely grazed pastures in Alabama, Georgia, Louisiana, and Mississippi were small or intermediate in plant type (Brink et al., 1999).

Persistence of white clover is not determined solely by survival of individual plants, but by persistence of the stand. White clover stands may persist under cool, moist conditions or climates by stolon survival. In climates with periods unfavorable (i.e., hot, dry) for white clover growth, stands are maintained by hard seed production and germination when conditions are favorable for white clover growth (Harris, 1987). Both of these survival strategies only succeed under appropriate management and environmental conditions. Promotion of one survival strategy is often at the expense of the other. Each stolon node can form either a lateral stolon (promoting vegetative persistence) or a flower (promoting seed production) but not both (Thomas, 1987; Pederson, 1995). Stolon density is closely related to vegetative persistence, while profuse flowering and seed production are inversely related to plant persistence (Gibson, 1957; Piano and Annicchiarico, 1995).

Seed production differs among cultivars and among types of white clover. Generally, small-type white clovers produce more flowers than large types, but large types have more seeds per flower than small types in temperate climates (Hollington et al., 1989; Marshall et al., 1989; Williams et al., 1998). Differences among cultivars have been noted not only for seed yield, but also for most seed yield components (Connolly, 1990; Williams et al., 1998). Usually, studies of white clover seed production have taken place in areas of commercial seed production (Connolly, 1990; Oliva et al., 1994) rather than in areas of pasture use. Seed production potential of white clover for reseeding under pasture conditions has not been sufficiently evaluated.

In a four-state trial, Brink et al. (1999) reported that under continuous stocking naturalized populations had greater stolon length, stolon branching, and nodal rooting than cultivars. They also observed that flower and volunteer seedling production was greater for naturalized populations than cultivars. The objective of this study was to quantify and compare the relative seed yield and seed yield components of white clover culti-

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**Table 1. White clover entries evaluated for seed production in a bermudagrass pasture.**

| Entries | Plant type | Origin | Characteristics (reference)† |
|---|---|---|---|
| **Cultivars** | | | |
| Grasslands Huia | Intermediate | New Zealand | Selected for yield and persistence in New Zealand (b,c) |
| Grasslands Prestige | Intermediate | New Zealand | Selected for sheep grazing systems in New Zealand (c) |
| Louisiana S-1 | Intermediate | LA | Selected for heat and drought tolerance; prolific flowering (b,c) |
| Osceola | Large | FL | Selected for flower production and persistence; medium flowering (b,c) |
| Regal | Large | AL | Selected for summer production and persistence; low flowering (b,c) |
| **Germplasms** | | | |
| Brown Loam Syn 2 | Large | MS | Selected for drought tolerance and summer survival (c) |
| SRVR | Large | SC, NC, VA | Selected for multiple virus resistance (c) |
| **Naturalized (pasture collected) populations** | | | |
| Alabama | Small | AL | Collected from 10 Alabama pastures (a,d) |
| North GA | Small | GA | Collected from two pastures in Eatonton, GA (a,d,f) |
| South GA | Small | GA | Collected from a pasture in Tifton, GA (a,d) |
| Homer LA | Small | LA | Collected from two pastures in Homer, LA (e) |
| Starkville MS | Small | MS | Collected from five pastures near Starkville, MS (a,d) |
| Pontotoc MS | Small | MS | Collected from four pastures near Pontotoc, MS (a,d) |
| Raymond MS | Small | MS | Collected from three pastures near Raymond, MS (a,d) |

† Letter in parentheses is the reference source of characteristic information: a = Brink et al., 1999; b = Caradus, 1986; c = Caradus and Woodfield, 1997; d = Pederson and Brink, 1997; e = B. C. Venuto, personal communication, 1994; and f = Bouton et al., 1998.

vars and germplasms with those of naturalized populations under pasture conditions following termination of grazing. This should be considered potential seed production in a pasture since no grazing was conducted on the plots following the initiation of flowering.

## MATERIALS AND METHODS

Fourteen cultivars, germplasms, and naturalized populations were used in this study (Table 1). The seven cultivars and germplasms were intermediate- to large-type white clover and the seven naturalized populations were small to intermediate type. Seed of naturalized populations was collected from closely grazed pastures in Alabama, Georgia, Louisiana, and Mississippi. Seed of each population was increased by caged crosses using honey bees, *Aphis mellifera* L.

One hundred plants of each entry were started from seed and grown in the greenhouse for 8 wk. The plants were space-planted into a common bermudagrass pasture at the Leveck Animal Research Center, Mississippi State, MS, on a Savannah fine sandy loam on 8 Nov. 1995 and 30 Oct. 1996 in separate, adjacent experimental sites. Fertility of the entire pasture was maintained as reported by Brink et al. (1999). Each plot was 1 m$^2$ with 25 plants evenly spaced 25 cm apart in a five by five grid. There were 1.5 m between each plot and each replicate. Plots were grazed by cattle (*Bos taurus*) each year from early March to early May when the white clover plants began to flower. The plots were continuously stocked with cattle to maintain a sward height of 5 cm (Forage and Grazing Terminology Committee, 1992). The cattle were then removed for the duration of the study. All white clover plants were allowed to flower and set seed naturally with no additional defoliation. Native pollinators (honey bees and bumble bees [*Bombus* spp.]) were available in adequate numbers for pollination. No additional insect pollinators were used to improve seed production at the study site. The studies were terminated each year when all flower heads had ripened by early August. The plots used in this study were separate from those evaluated by Brink et al. (1999) at Mississippi State for growth and persistence of the same 14 white clover entries under continuous stocking.

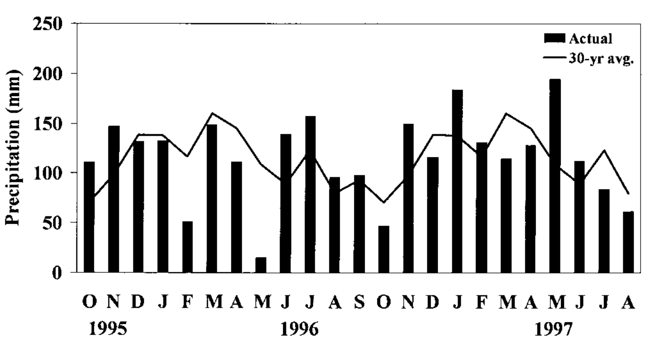


**Fig. 1. Actual and 30-yr average monthly precipitation at Mississippi State, MS during the duration of the experiment.**

Data were collected within duplicate 15 by 15 cm quadrats in each plot on six dates from 8 May to 12 Aug. 1996 and 19 May to 6 Aug. 1997. The quadrats were randomly placed in the plots on the first sampling date. These locations were marked and later samples were taken with the quadrats placed at the same locations within the plots. Data were taken on number of flower heads with at least one floret completely open, number of ripe seed-bearing flower heads, number of seeds, and seed weight. Seed-bearing flower heads were harvested by hand, dried in paper bags in the greenhouse, and threshed by hand to prevent scarification of seed.

The experimental design was a randomized complete block with four replicates. Data were analyzed by analysis of variance (SAS Institute, 1990). Means were compared by Fisher's protected least significant difference (LSD). Unless otherwise noted, the 0.05 level of probability was used to determine differences. Pearson product-moment correlations were computed using entry means (SAS Institute, 1990).

## RESULTS

During the winter and spring months of both years of this study, precipitation was generally close to the 30-yr average. However, during the flowering and seed maturation period, precipitation varied between the 2 yr of the study (Fig. 1). In 1996, May was a dry, warm month with 15 mm of rainfall and temperatures averaging above normal (temperature data not shown). Rainfall was above normal the rest of the flowering and seed-maturation season. In 1997, May was wet and cool with 194 mm of rainfall and temperatures averaging below normal. July and August had less rainfall than normal. These environmental factors resulted in more flowers, more seed-bearing flowers, and greater seed yields by white clover in 1996 than 1997. Interactions ($P < 0.01$)

**Table 2. Total and seed-bearing flower production and ratio of seed-bearing flowers to total flowers of white clover cultivars, germplasms, and naturalized populations grown in a bermudagrass pasture.**

| Entries | 1996 Flowers† | 1996 Seed-bearing flowers‡ | 1996 Ratio§ | 1997 Flowers† | 1997 Seed-bearing flowers‡ | 1997 Ratio§ |
|---|---|---|---|---|---|---|
| | no. m$^{-2}$ | no. m$^{-2}$ | % | no. m$^{-2}$ | no. m$^{-2}$ | % |
| Cultivars and germplasms | | | | | | |
| Louisiana S-1 | 1329 | 1135 | 85.4 | 1065 | 963 | 90.4 |
| Osceola | 1103 | 511 | 46.3 | 630 | 387 | 61.5 |
| Prestige | 893 | 791 | 88.6 | 404 | 312 | 77.4 |
| Brown Loam Syn 2 | 635 | 355 | 55.9 | 382 | 248 | 64.7 |
| SRVR | 527 | 258 | 49.0 | 753 | 640 | 85.0 |
| Huia | 490 | 420 | 85.7 | 457 | 323 | 70.7 |
| Regal | 447 | 210 | 47.1 | 371 | 280 | 75.7 |
| Mean | 813 | 526 | 64.7 | 580 | 450 | 77.6 |
| Naturalized populations | | | | | | |
| Raymond MS | 3061 | 2529 | 82.6 | 1248 | 1168 | 93.5 |
| Homer LA | 2674 | 2453 | 91.7 | 1270 | 1205 | 94.9 |
| South GA | 2340 | 2034 | 86.9 | 1377 | 1264 | 91.8 |
| North GA | 2313 | 1975 | 85.3 | 1549 | 1404 | 90.6 |
| Starkville MS | 2125 | 1840 | 86.6 | 1345 | 1205 | 89.6 |
| Pontotoc MS | 2125 | 1802 | 84.8 | 1286 | 1200 | 93.3 |
| Alabama | 2082 | 1625 | 78.0 | 1307 | 1168 | 89.3 |
| Mean | 2393** | 2037** | 85.1** | 1340** | 1230** | 91.8** |
| LSD 0.05 | 627 | 522 | 17.8 | 280 | 275 | 14.9 |

** Naturalized population mean significantly greater than cultivar and germplasm mean by *F* test at the 0.01 level.
† Total number of flowers from six harvests each year.
‡ Number of flower heads bearing seeds when harvested.
§ Ratio of number of seed-bearing flowers to total number of flowers.

were observed between year × entries for flowers, seed-bearing flowers, seed yield, and seeds per flower. Therefore, all data were analyzed and will be presented separately for each year.

The seven naturalized populations had more flowers than all cultivars in 1996 and six of seven cultivars in 1997 (Table 2). Louisiana S-1 was the only cultivar to have flower numbers similar to the naturalized populations, and then only in 1997. Cultivars had 34% in 1996 and 43% in 1997 as many flowers as the naturalized populations. The number of seed-bearing flowers followed a similar trend, with the seven naturalized populations having more seed-bearing flowers than all cultivars except Louisiana S-1 in both years (Table 2). Cultivars had 26% in 1996 and 37% in 1997 as many seed-bearing flowers as the naturalized populations.

Naturalized populations not only produced more flowers, but also had a greater percentage of flowers that produced seed both years (Table 2). White clover flowers that have no seed usually dry up and drop to the ground prior to harvest. Most flowers of the naturalized populations produced seed. A clear difference due to plant type was noted in 1996. The three intermediate cultivars (Louisiana S-1, Huia, and Prestige) had seed-bearing/total flower ratios equivalent to the naturalized populations, while the large-type cultivars had lower seed-bearing/total flower ratios. In 1997, Prestige and Louisiana S-1 were again equivalent to most of the naturalized populations, as were the large-type SRVR and Regal. There was no difference among the naturalized populations for ratio of seed-bearing to total flowers either year.

The difference in flower production between cultivars and naturalized populations was fairly consistent across the entire flower production season. Greater numbers of flowers were found for populations than for cultivars in all six harvests in 1996 and four of six harvests in 1997 (Fig. 2). Maximum flower production for the naturalized populations was earlier each year (10 June 1996 and 29 May 1997) than the cultivars (3 July 1996 and 26 June 1997).

Seed production of the seven naturalized populations was greater than that of all cultivars except Louisiana S-1 in 1996 (Table 3). In 1997, six of seven naturalized populations had greater seed production than all cultivars except Louisiana S-1, SRVR, and Osceola. The Homer, LA population only had greater seed production in 1997 than the two New Zealand cultivars, Huia and Prestige. Cultivars averaged 27% as much seed as the populations in 1996 and 43% as much in 1997. Most of the seed ripened and was harvested between 10 June and 8 July each year (Fig. 3). Naturalized populations had greater seed yields ($P < 0.01$) than cultivars on the third and fourth harvest dates in 1996 (10 June and 3 July) and fourth and fifth harvest dates in 1997 (26 June and 8 July; data not shown). No differences between

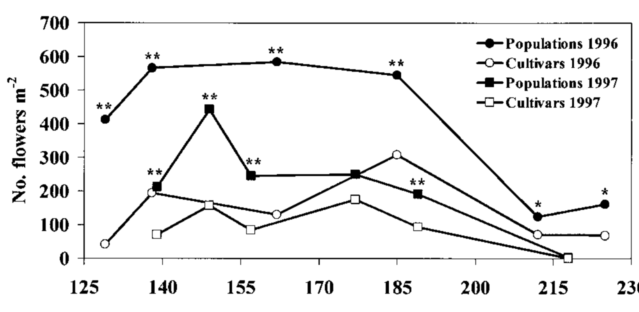


**Fig. 2. Number of flowers per square meter of seven naturalized white clover populations and seven cultivars and germplasms in 1996 and 1997 sampled at six different days of the year. *, ** Flower numbers of the naturalized populations were greater than those of the cultivars and germplasms at the 0.05 and 0.01 level, respectively.**

**Table 3. Total seed yield, number of seeds per seed-bearing flower, and 1000-seed weight of white clover cultivars, germplasms, and naturalized populations grown in a bermudagrass pasture.**

| Entries | 1996 Seed yield | 1996 Seeds per flower† | 1996 1000-seed weight | 1997 Seed yield | 1997 Seeds per flower† | 1997 1000-seed weight |
|---|---|---|---|---|---|---|
| | $g\ m^{-2}$ | no. | g | $g\ m^{-2}$ | no. | g |
| **Cultivars and germplasms** | | | | | | |
| Louisiana S-1 | 35.4 | 63.2 | 0.502 | 26.9 | 59.2 | 0.468 |
| Osceola | 17.1 | 71.8 | 0.480 | 16.6 | 87.8 | 0.465 |
| Prestige | 13.0 | 42.2 | 0.409 | 3.6 | 26.7 | 0.421 |
| Brown Loam Syn 2 | 8.5 | 52.9 | 0.451 | 8.9 | 79.4 | 0.474 |
| SRVR | 6.9 | 55.8 | 0.474 | 18.4 | 63.8 | 0.450 |
| Huia | 8.0 | 56.5 | 0.460 | 4.1 | 31.7 | 0.422 |
| Regal | 4.3 | 46.4 | 0.451 | 7.7 | 61.7 | 0.437 |
| Mean | 13.3 | 55.6 | 0.461 | 12.3 | 58.6 | 0.448 |
| **Naturalized populations** | | | | | | |
| Raymond MS | 56.9 | 45.3 | 0.483 | 22.5 | 43.6 | 0.463 |
| Homer LA | 61.1 | 53.3 | 0.472 | 17.7 | 30.0 | 0.479 |
| South GA | 50.0 | 55.5 | 0.449 | 23.6 | 41.6 | 0.456 |
| North GA | 42.9 | 46.9 | 0.467 | 40.6 | 64.2 | 0.457 |
| Starkville MS | 50.1 | 60.7 | 0.450 | 25.1 | 45.6 | 0.453 |
| Pontotoc MS | 47.7 | 56.5 | 0.470 | 34.6 | 64.5 | 0.454 |
| Alabama | 38.2 | 53.0 | 0.450 | 35.9 | 70.2 | 0.436 |
| Mean | 49.5** | 53.0 | 0.463 | 28.6** | 51.4 | 0.457 |
| LSD 0.05 | 15.4 | NS | 0.040 | 11.8 | 22.3 | NS |

** Naturalized population mean significantly greater than cultivar and germplasm mean by *F* test at the 0.01 level.
† Number of seeds per seed-bearing flower heads.

populations and cultivars were found at any other date. Accumulated seed yields were greater ($P < 0.01$) for the naturalized populations than the cultivars on the last four harvests in 1996 and the last three harvests in 1997 (Fig. 3).

Few differences were noted between cultivars and naturalized populations for number of seeds per flower head and 1000-seed weight (Table 3). In 1996, Louisiana S-1 had greater 1000-seed weights than four of six cultivars and three of seven populations, but no differences were noted in 1997. In 1997, Osceola had more seeds per flower head than all cultivars except Brown Loam Syn. 2 and all populations except Alabama. No differences among entries for number of seeds per flower were noted in 1996.

Number of flowers, number of seed-bearing flowers, and seed yield were all highly correlated in both years (Table 4). Seed yield was correlated with number of seeds per flower in 1997, but not in 1996. Seed yield ($g\ m^{-2}$) was highly correlated both years ($r = 0.99$, $P < 0.01$) with total number of seeds per square meter. All

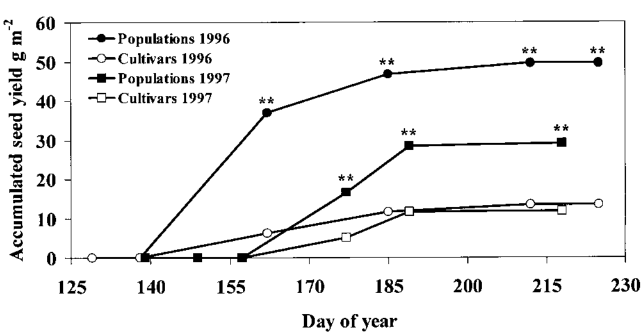


**Fig. 3. Accumulated seed yield ($g\ m^{-2}$) of seven naturalized white clover populations and seven cultivars and germplasms in 1996 and 1997 sampled at six different days of the year. ** Accumulated seed yields of the naturalized populations were greater than those of the cultivars and germplasms at the 0.01 level.**

relationships discussed here regarding seed yield in grams per square meter also held true for total number of seeds produced. There were no other correlations between any of the other characters.

## DISCUSSION

Most research interest in white clover seed production has been strictly for commercial uses, with little interest expressed in pasture seed production under grazing. White clover commercial seed production differs a great deal from seed produced within a pasture environment. In the USA, commercial seed production of white clover is conducted mainly in California using pure clover stands under irrigation, with a dry seed maturation period, long photoperiod, and adequate honeybee pollination (Marble, 1990; Pederson, 1995). Excessive vegetative growth is minimized to favor flower production (Zaleski, 1970; Oliva et al., 1994). In the southeastern USA, white clover is grown in mixed-species pastures usually containing <30% white clover. There is usually no irrigation, and conditions may be quite humid during seed pollination and maturation. Pollination is dependent upon wild pollinators, the photoperiod is short, and vegetative growth is managed strictly for animal production. Coupled with the influence of grazing on flower and seed production, it is reasonable to assume that seed production of white clover as a method of maintaining stands in the southeastern USA is not very important.

Components of white clover seed yield are flowers per unit area, florets per flower, seeds per floret, and seed weight (Connolly, 1990; Oliva et al., 1994). Most studies estimate the number of florets per flower and seeds per floret from relatively small samples of 5 to 10 flowers per plot (Hollington et al., 1989; Connolly, 1990; Annicchiarico, 1993; Oliva et al., 1994; Williams et al.,

**Table 4. Correlations between seed production characters for white clover cultivars, germplasms, and naturalized populations grown in a bermudagrass pasture in 1996 (above diagonal) and 1997 (below diagonal).**

| | Flowers | Seed-bearing flowers† | Seed yield | Seeds per flower‡ | 1000-seed weight |
|---|---|---|---|---|---|
| Flowers (no.) | | 0.98** | 0.92** | −0.12 | 0.15 |
| Seed-bearing flowers (no.) | 0.99** | | 0.93** | −0.16 | 0.12 |
| Seed yield (g m$^{-2}$) | 0.82** | 0.78** | | 0.13 | 0.22 |
| Seeds per flower (no.) | 0.02 | −0.06 | 0.47** | | 0.23 |
| 1000-seed weight (g) | −0.01 | −0.01 | −0.02 | −0.09 | |

** Correlation coefficient significantly greater than zero at the 0.01 level.
† Number of flower heads bearing seeds when harvested.
‡ Number of seeds per seed-bearing flower heads.

1998). In this study, seeds per flower was computed directly by dividing the total number of seeds per area by the total number of flowers, rather than estimating florets per flower and seeds per floret from samples. Many studies have reported greater differences among cultivars (Hollington et al., 1989; Annicchiarico, 1993; Williams et al., 1998) and greater genetic variability (Hill et al., 1989) for the number of florets per flower than the number of seeds per floret. Seed yield components tend to compensate for each other, such that decreasing the number of florets per flower results in an increased number of seeds per floret (Clifford, 1985; Thomas, 1987), and reducing the flower number results in an increase in the number of seeds per flower (Binek, 1983). The key determinant of seed yield in this study as well as in many others (Zaleski, 1970; Clifford, 1985; Hollington et al., 1989; Domingues et al., 1991; Oliva et al., 1994) was the number of flowers per square meter.

The seeding rate of white clover is normally 2.2 to 3.4 kg ha$^{-1}$ (Ball et al., 1991) or in units used in this study 0.22 to 0.34 g m$^{-2}$. The range of seed production observed for cultivars (4.3–35.4 g m$^{-2}$ in 1996 and 3.6–26.9 g m$^{-2}$ in 1997) and naturalized populations (38.2–61.1 g m$^{-2}$ in 1996 and 17.7–40.6 g m$^{-2}$ in 1997) was far in excess of that needed to establish a white clover stand the following year. However, when germination tests were conducted on the nonscarified seed immediately after harvest in this study, both cultivars and populations averaged 97.5 ± 2.0% hard seed. Some of this hard seed would survive in the soil and be available for germination in the future under favorable environmental conditions. With only 2.5% soft seed, the amount of soft seed available for germination averaged 0.32 g m$^{-2}$ (range 0.09–0.88 g m$^{-2}$) for cultivars and 0.98 g m$^{-2}$ (range 0.44–1.53 g m$^{-2}$) for naturalized populations. This seed amount would be the potential amount of new seed available for natural reseeding. Additional reseeding could occur from hard seed that softened and germinated in the first fall. For example, Chapman and Anderson (1987) found 75% hard seed at harvest in a ‘Grassland Huia’ and small-leaved naturalized population in New Zealand. Of these hard seed, 22% were found to germinate within the first 7 mo. In our study, no measurements were made of the proportion of hard seed that would soften and germinate in the first fall.

Under pasture conditions, the number of white clover seeds that produce seedlings is greatly reduced by a number of factors including animal grazing and trampling, insect feeding, seed decomposition, grass shading and competition, lack of soil disturbance, and unfavorable environmental conditions (Barrett and Silander, 1992). For example, Chapman and Anderson (1987) reported that 94% of white clover flowers were removed by sheep (*Ovis aries*) grazing in New Zealand pastures. Differences have been noted among white clover plant types for flowering and reseeding under grazing. Under continuous cattle stocking, greater flower numbers and seedling recruitment were observed for naturalized populations than for cultivars (Brink et al., 1999). Comparing the 1996 flowering data of the present study with unpublished data from the study of Brink et al. (1999) involving the same entries also at Mississippi State, 70% of the flowers of both cultivars and populations were removed by continuous cattle grazing in May 1996. In July only 32% of the population flowers and 84% of the cultivar flowers were removed by grazing. Therefore, not only do the populations produce more flowers, but the low growth stature of the small-type plants probably enables a greater proportion of the flowers to occur below the level of cattle grazing in the summer.

The greater seed production of naturalized populations compared with cultivars suggests the potential for similar reseeding differences in pastures. Excessive seed production by naturalized populations and persistence under intensive grazing would enable them to have a greater opportunity to reseed even under unfavorable conditions. Also, the larger quantity of hard seed contained within the seed bank would give naturalized populations a better opportunity than cultivars to reseed even after years of unfavorable environmental conditions. The limited seed production of cultivars reduces their opportunity for reseeding both in the immediate future from soft seed and in the more distant future from hard seed. All of these factors contribute to the dominance of small-type white clover in closely grazed pastures in the southeastern USA.

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